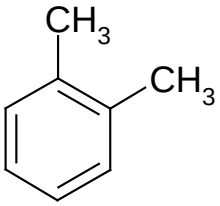
NTP Research Concept: Xylenes

Project Leader

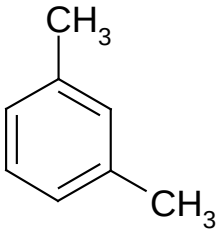
Matthew D. Stout, PhD, DABT, NIEHS/DNTP, Program Operations Branch

Background and Rationale

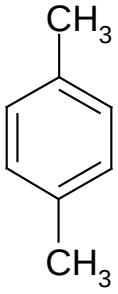
Xylenes are alkylated monoaromatic hydrocarbons produced in high volumes and present in all environmental media. Xylenes have been nominated for NTP testing on multiple occasions by different orgnizations and for a variety of toxicological endpoints. The NTP previously conducted prechronic and chronic toxicity and carcinogenicity studies of mixed xylenes in rats and mice by oral gavage (http://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr327.pdf). Despite a relatively robust database, significant data gaps and dataset limitations remain for these ubiquitous compounds as evident from information included in an Agency for Toxic Substances and Disease Registry (ATSDR) Toxicological Profile (2007) (http://www.atsdr.cdc.gov/toxprofiles/tp71.pdf), an Environmental Protection Agency (EPA) Integrated Risk Information System (IRIS) assesment (2003) (http://www.epa.gov/iris/subst/0270.htm), and documentation from the US EPA's Voluntary Children's Chemical Evaluation Program (VCCEP) (http://www.epa.gov/oppt/vccep/pubs/chem7.html; http://www.tera.org/Peer/VCCEP/xylenes/xylenesWelcome.html). Overall, there are multiple toxicity and carcinogenicity database deficiencies for xylenes, but disagreement over which deficiencies warrant further testing. This research will attempt to address certain of these outstanding database deficiencies.



o-xylene



m-xylene



p-xylene

Mixed xylenes (CAS RN 1330-20-7) are produced commercially from petroleum in an approximate isomeric ratio of 40-44% *m*-xylene, 20% each *o*-xylene and *p*-xylene, and 15-20% ethylbenzene, with other minor contaminants such as toluene. Nearly all mixed xylene is produced as a catalytic reformate of petroleum. Recent data indicate that the U.S. annual production is >8 billion pounds/year (http://java.epa.gov/oppt_chemical_search/). Approximately 70% of xylenes are used in the production of ethylbenzene and individual xylene isomers, while approximately 30% is used in solvents, paints/coatings, and in gasoline; xylene is a member of "BTEX" (benzene, toluene, ethylbenzene and xylene), which are the most soluble compounds in gasoline and are often used as markers of gasoline contamination in soil and groundwater. Individual xylene isomers are used

primarily for internal industrial operations, as solvents and reaction intermediates. Occupational and consumer exposure to xylenes is primarily via inhalation, through emissions from industrial sources, such as petroleum refineries and chemical plants; automobile exhaust; and volatilization from use as solvents. In addition, xylenes are found in soil, water, and some foods as a result of contamination due to leaching or spills. Overall, releases into air are 2-3 orders of magnitude greater than releases into water or soil.

The disposition of xylenes and toxicity and carcinogenicity of xylenes in humans and in animals have recently been reviewed by ATSDR, the U.S. EPA and Office of Environmental Health Hazard Assessment (*OEHHA*) (http://www.oehha.ca.gov/prop65/hazard_ident/pdf_zip/092812XyleneHID.pdf).

The disposition and major toxicities of xylenes are similar between humans and animals. Xylenes are rapidly absorbed, metabolized primarily by the liver, well distributed, in particular to lipid rich tissues, and eliminated primarily in the urine following glycine conjugation or as parent compound in expired air. A major distinction between routes is that inhalation exposure allows direct contact with the respiratory tract following inhalation exposure and distribution to tissues without first pass metabolism. The primarily toxicities of xylenes involve the respiratory tract and central nervous system. Much of the toxicity data generated on xylenes involves co-exposure to ethylbenzene.

The EPA IRIS Assessment indicated that data in both humans and animals are inadequate to evaluate potential associations between xylene exposure and cancer. Xylenes are also classified by IARC as Group 3, *not classifiable as to its carcinogenicity to humans* (http://monographs.iarc.fr/ENG/Monographs/vol71/mono71-58.pdf). In humans, multiple studies suggest possible associations between exposure to mixed xylenes and cancer. However, these studies are limited primarily by co-exposures to other chemicals. In addition, no inhalation studies to assess the potential carcinogenicity of mixed xylenes in animals have been conducted. The NTP previously conducted toxicity and carcinogenicity studies of mixed xylenes via oral gavage in rats and mice (http://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr327.pdf). Exposure to mixed xylenes did not result in carcinogenic activity in these studies; however, these studies were not adequate to assess direct effects of mixed xylene on the respiratory tract. NTP inhalation studies of structurally related compounds, including cumene (isopropylbenzene; http://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr542.pdf) and ethylbenzene (http://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr466.pdf) demonstrated carcinogenic activity in male and female rats and mice. Sites in these studies were the lung (ethylbenzene and cumene) and nose (cumene only); lung tumors in male mice may have been related to exposure to styrene following exposure via gavage (http://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr185.pdf). The NTP Report on Carcinogens lists styrene as *reasonably anticipated to be a human carcinogen* and the NTP recommends that cumene be listed *as reasonably anticipated to be a human carcinogen*. In contrast, toluene was not carcinogenic in NTP inhalation studies in rats

and mice (http://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr371.pdf) at the concentrations tested.

In addition to carcinogenicity, reviews of the available toxicity data by ATSDR, US EPA and OEHHA have demonstrated that there are also limitations in the data sets for reproductive toxicity, developmental toxicity, and neurotoxicity. In humans, studies aimed at evaluating reproductive and developmental effects of mixed xylenes, although suggestive of associations, are limited by co-exposures to other chemicals. There are very limited data to evaluate reproductive toxicity in rodents, as the dataset consists primarily of one single generation inhalation study in which animals were exposed from pre-mating through lactation. There were no effects on reproductive parameters in this study. In other studies, there is conflicting evidence on effects of xylenes on male reproductive tissue weights and sperm. A number of developmental toxicity studies have been conducted, primarily via the inhalation route. Although skeletal abnormalities and delayed ossification were observed in many of these studies, exposure to concentrations that resulted in these effects frequently also resulted in maternal toxicity. Overall, the developmental dataset is limited by major differences in design among studies, a lack of guideline studies, a lack of litter-based statistics, and a lack of reporting of maternal toxicity data. Several reports aimed at examining neurobehavioral effects following developmental exposures have demonstrated limited evidence of effects at similar concentrations to those inducing neurologic effects in adult animals. For example, in one study, non-enriched animals, but not enriched animals, demonstrated a delay in finding a platform in the Morris Water maze at one time point. These effects occur at similar exposure concentrations to those that produced neurological effects in adult animals.

Given the magnitude of production, the use patterns in occupational and consumer settings, the observed effects of xylene and structurally related compounds in the respiratory tract, and the limitations in the available data sets for toxicity and carcinogenicity in humans and animals, further studies to characterize the toxicity and carcinogenicity of mixed xylenes by the inhalation route are warranted.

Key Issues

Most mixed xylene formulations contain 15-20% ethylbenzene, which has been demonstrated to be carcinogenic in rats and mice following inhalation exposure (NTP). Given the carcinogenicity of ethylbenzene and that the human dataset for mixed xylenes is limited by co-exposures to other compounds, a test agent free of ethylbenzene is needed in order to allow assessment of the toxicity or carcinogenicity of xylenes. Inhalation studies to evaluate the subchronic and chronic toxicity as well as endpoints aimed at assessing the potential for xylenes to induce reproductive toxicity, developmental toxicity, and neurotoxicity are needed. There are advantages to generating data on each of these endpoints under the same exposure scenario, most notably efficiencies in chemistry, exposure system design and generation/monitoring, and animal husbandry; and the ability to make direct dose-response comparisons across multiple endpoints. Despite these advantages, there are logistical and technical challenges in conducting developmental exposures via inhalation, notably the exposure

and husbandry of dams and offspring during the lactation phase and the time constraints resulting from performing exposures, husbandry of dams/offspring and functional assessments within the same day.

Specific Aims

1) Obtain a high purity mixture of *o*-, *m*-, and *p*-xylene free of ethylbenzene for use as a test agent in the conduct of toxicity and carcinogenicity studies (aims 2 and 3).

2) Evaluate the subchronic toxicity and chronic toxicity and carcinogenicity of xylenes following whole body inhalation exposure.

3) Evaluate the developmental toxicity, reproductive toxicity, and neurotoxicity of xylenes following whole body inhalation exposure.

Proposed Approach

1) Commercially available mixed xylene preparations contain *o*-, *m*-, and *p*-xylenes, ethylbenzene (~15-20%) and other impurities (e.g., toluene). Several commercially available preparations of mixed xylenes will be analyzed to determine representative ratios of individual xylene isomers. Individual isomers at a high purity will be procured and blended to an isomeric ratio representative of commercially available preparations of mixed xylenes to achieve a high purity mixture of *o*-, *m*-, and *p*-xylene that does not contain ethylbenzene. This test agent will be used for studies outlined below.

2) Conduct subchronic and chronic whole body inhalation toxicity and carcinogenicity studies of xylenes using high purity mixture of *o*-, *m*-, and *p*-xylene without ethylbenzene.. Exposure concentrations for the chronic studies will be selected following a review of the subchronic data. Short-term inhalation studies on individual isomers may also be included for individual isomers obtained as described above in order to determine if there are differences in toxicity among these isomers.

3) In conjunction with the whole body inhalation toxicity and carcinogenicity studies described above, additional animals will be exposed to generate data on reproductive, developmental, and neurotoxicity endpoints. Selection of endpoints will be tailored to provide robust data in the context of the logistical challenges outlined above.

Significance and Expected Outcome

Although the production, use, and human exposure potential to xylenes is significant, the human and animal datasets are have significant weaknesses, including co-exposures to other chemicals. This research will generate data to evaluate whether carcinogenic activity or reproductive, developmental, and neurotoxic effects can be associated with inhalation exposure to a highly pure mixture of *o*-, *m*- and *p*-isomers of xylene. These studies would fill significant data gaps and permit a better understanding of the toxic and carcinogenic effects of xylenes. In addition, the generation of data on a

variety of toxicities under similar exposure conditions will allow for dose-response comparisons across multiple endpoints to examine relative sensitivities and will improve confidence in the risk assessments of xylenes.